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Study the correlation between HAMP gene polymorphism (582A/G) and beta thalassemia in Kirkuk city

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Abstract

The current study aimed to Study the correlation between HAMP gene polymorphism (-582A/G) and beta thalassemia. 150 subjects with thalassemia were taken from both Azadi Teaching Hospital and Al Jumhuri Hospital from November 2021 to April 2022. The experimental work was carried out in private laboratories in Kirkuk, Iraq. The volunteers in the current study were divided as follows: 50 healthy volunteers as a control group. 150 thalassemia patients as a second group. The results of Polymorphism of the hepcidin gene (HAMP) in patients with anemia of CKD showed that the AA allele was 68 (68%), the AG allele 21 (21%) and the GG allele was 11 (11%) in 100 patients, in While in the group of healthy people, the percentage of the AA allele was 37 (76%), the percentage of the AG allele was 12 (24%), and the percentage of the GG allele was 1 (2%), and thus the results showed that the A allele is more common in patients with anemia resulting from for chronic kidney disease.

Keywords

Beta thalassemia, HAMP gene polymorphism, hepecidin.

Although thalassemia is common in the countries surrounding the Mediterranean Sea, especially Italy and Greece, it is present in any region where malaria is endemic (Middle East, India and Northeast Asia) because the heterozygous condition of thalassemia provides a protective effect against malaria [1]. Thalassemia has become worldwide as a result of migration from its first discovery areas: Greece, Italy, Sardinia, Cyprus, and Turkey [2]. Studies have shown that the gene frequency of beta thalassemia in these cities is in the range of (5-25%) [3]. Studies showed the spread of the disease in many countries of the world, including Malaysia [4], Germany [5], Iran [6] and the United States [7]. As for the Arab countries, the disease is registered in the Arab cities located on the Mediterranean basin, as well as many other Arab countries, including Saudi Arabia, Jordan, Egypt, Lebanon, and Yemen [8]. As for Iraq, studies have mentioned the spread of the disease in a number of governorates of the country, and its spread constitutes (5.4%-5%) of the total Iraqi society [9]. And these disorders are more common in specific ethnic groups, which shows the effect of race through the investigation of communities [10], and the following table shows the societies that have an increased risk of thalassemia. Human hepcidin, a 25-amino-acid peptide made by hepatocytes, may be a new mediator of innate immunity and the long-sought iron-regulating hormone. Hepcidin synthesis is greatly stimulated by inflammation or by iron overload. Evidence from transgenic mouse models indicates that hepcidin is the dominant negative regulator of iron uptake in the small intestine, transport of iron across the placenta, and release of iron from macrophages. The major role of hepcidin was confirmed by the presence of nonsense mutations in the hepcidin gene, homozygous in affected organs, in two families with juvenile acute hemochromatosis. Discovery of hepcidin and its

role in iron metabolism could lead to new therapies for hemochromatosis and inflammatory anemia [11-14].

Materials & Methods

Samples collection

150 subjects with thalassemia were taken from both Azadi Teaching Hospital and Al Jumhuri Hospital from November 2021 to April 2022. The experimental work was carried out in private laboratories in Kirkuk, Iraq. The volunteers in the current study were divided as follows:

- 50 healthy volunteers as a control group.
- 150 thalassemia patients as a second group.

Collection of blood samples

Blood samples were collected by well-trained nurses from each patient. 5 ml of venous blood samples were obtained from each person and divided into 1 ml in an EDTA tube and 4 ml in a normal tube.

Preparation of sample

After incorporating 5 ul of purified DNA for electrophoresis with 3 ul of loading buffer (Intron company/ Korea), the loading process into the gel's gaps is already currently in progress. A 70 V CM2 electric charge was applied for 1-2 hours till the tincture reached the opposite side d the gel. The gel was examined by a source of UV light with a wavelength of 336 nm after already being put in a pool with 500 milliliters of DW and 30ul of red safe nucleic acid dye.

DNA isolation and genotyping

2 mL of blood collected in an tube containing EDTA for Extraction of DNA t o extract DNA, salting out has been utilized (Lahari et al. 1992). Appropriate amounts of Erythrocytes lysis buffer comprising Triton-X were put to the whole sample of blood to lyse the Erythrocytes and acquire the pellet. After lysing the pellet with Leucocyte lysis solution that contains 10% SDS, high molar concentrations of Sodium chloride were added sequentially to separate the protein fraction. After that, the DNA was isolated, resuspended in TE buffer, and stored at -200C until the PCR reaction has been performed. After that, ice-cold ethanol was then added. The polymorphism in IL-10 (1082 A/G) was investigated utilizing PCR amplification reaction amplification refractory mutation system method (ARMS PCR).

The primers

The primers were solubilized, disintegrated in free ddh20 to a final amount of 100 pmol/ul as a standard solution, and stored at -20 to prepare a work primer suspended concentration of 10 pmol/ul. IDT then performed an experiment with 10 ul of the standard solutions and 90 ul of free ddh water to achieve a final volume of 100 ul.

Table (1): the specific primer of HAMP (-582A / G)gene

Primers		Sequence (5'-3')	Product size
HAMP (-582A / G)	G allele Wildtype Primer	CTGACACTGGGAAAACACCG	205bp
	A allele Mutant type Primer	CTGACACTGGGAAAACACCA	
	Common Reverse Primer	CTCACTTCCCCATCGCCTAC	

Statistical analysis

Statistic evaluation Means and SE were used to express the results. using one-way analysis of variance to statistically analyze the data, ANOVA was used to analyze the data and find differences between the groups before and after the treatment. SPSS (SPSS 2003, SPSS Inc.) was used to analyze the data, and P 0.05 was considered statistically significant.

Results & Discussion

HAMP gene polymorphism (-582A/G)

For the detection of the HAMP gene polymorphism (-582A/G), the Fast Digest HindIII restriction enzyme (Fermentas-Thermo-USA) was used. The digests were exposed to 2.5% agarose gel and showed that the A allele that does not contain the HindIII restriction enzyme site was digested to 200bp, while the G allele was digested to 190bp and 90bp (Fig. 15). Polymorphism of the hepcidin gene (HAMP) in patients with anemia of CKD showed that the AA allele was 68 (68%), the AG allele 21 (21%) and the GG allele was 11 (11%) in 100 patients, in While in the group of healthy people, the percentage of the AA allele was 37 (76%), the percentage of the AG allele was 12 (24%), and the percentage of the GG allele was 1 (2%), and thus the results showed that the A allele is more common in patients with anemia resulting from for chronic kidney disease (Table 2).

Table (2): Genotype frequencies of the HAMP gene polymorphism (-582A/G)

Genotype	Control group	Patient group	Total	P value
AA	37(74%)	68(68%)	105 (70%)	0.062
AG	12(24%)	21(21%)	33(22%)	
GG	1(2%)	11(11%)	12(8%)	
Total	50(33.3%)	100(66.7%)	150(100%)	

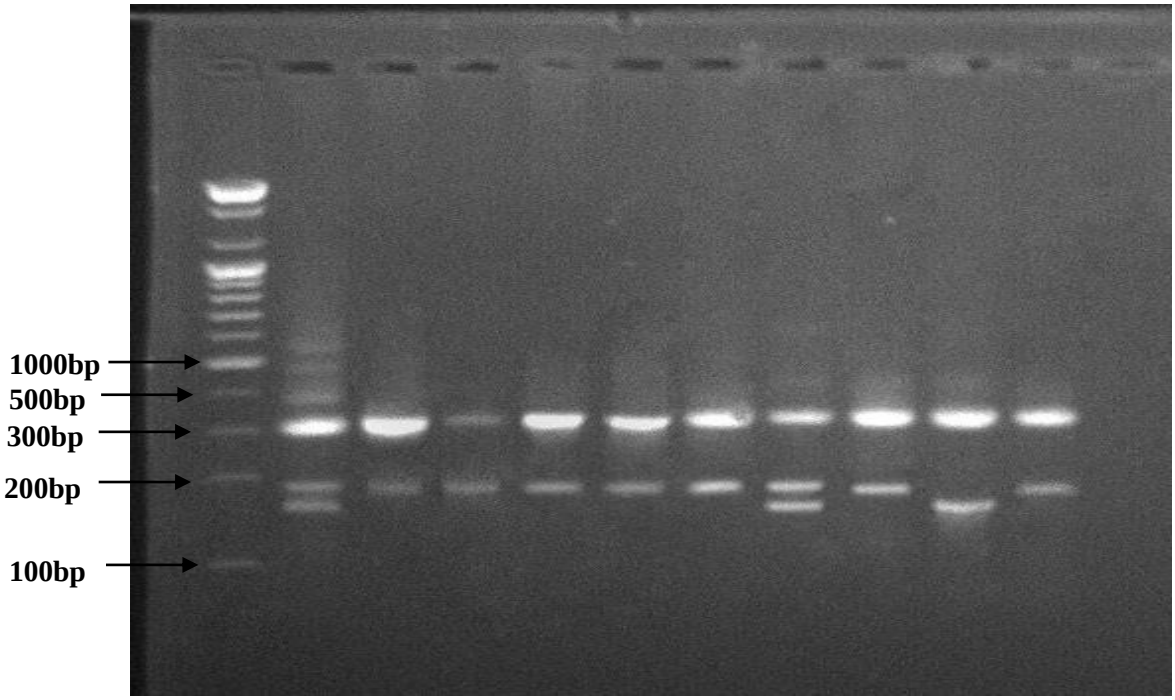

Figure 1: Agarose gel digestion of PCR products by HindIII restriction enzyme (HAMP).

DNA Ladder (100−1000 b.p). Lane 1, 2, 3, 4, 6, 7 : GG homozygote alleles (bands at 90 bp and190 bp). Lane 8, 9, 10, 11: AG heterozygote alleles (bands at 90 b.p, 190 b.p, and 200 b.p). Lane 5: AA homozygote alleles (band at 200 bp).

Hepcidin is an important regulator of iron homeostasis, which is involved in various metabolic pathways of iron metabolism [15]. It has been shown that some HAMP promoter region polymorphisms may reduce the expression of hepcidin and thus increase serum iron [16]. The present study showed that the genotype distribution and allele frequency of HAMP (-582A/G) in Iraq were compared in all volunteers (patients and healthy subjects), and no significant difference was observed between the studied groups. Besides, no significant difference was observed in the frequency of HAMP alleles (-582A/G) among the studied groups, where the P-value = (0.062). And when the patients were analyzed, a similar distribution of HAMP A/G genotype and allele frequency was found where the P-value = (0.238). , which indicates that HAMP A/G polymorphism is not involved in the pathophysiology of beta-thalassemia B in patients. The results of the current study are consistent with those of Nemeth and Ganz [11], who found no association between the HAMP genotype (-582A/G) and serum iron, ferritin concentration, and transferrin saturation levels. The current study also differs with Nemeth et al. [17], who found that the human HAMP gene polymorphism (-582A/G) has no effect on hepcidin transcription in normal conditions but has some effect in some pathophysiological conditions that require more hepcidin. However, from the results of the current study, it is unlikely that HAMP hepcidin (-582A/G) genetic variations play a significant role in genetic predisposition to ACKD, and the conflicting results may be due to different reasons such as subject demographic features and different lifestyle, volume plays The sample also played an important role in the difference between the results of the current study and some studies in Iraq and the world.

Parajes et al [18] found that the genotype (-582A/G) is in E-box 1 with a conserved sequence of CANNTG. E-box 1 is a responsive element of primary catalytic factors 1 and 2 (USF1/USF2) and

cMyc/Max heterodimers. When A is replaced by G, the transcription factors will not be able to adequately bind the E-box resulting in reduced transcription of the HAMP gene. On it, serum ferritin level was assessed and all patients with GG genotype had ferritin above 1000 ng/mL but no association was found between this SNP and serum ferritin level ($p = 0.12$) This could be explained by Our small sample size. It should be noted that although the research on this topic is not extensive, the results of the current study are not consistent with those presented by other researchers. For example, Andreani et al. [19] showed. that SNP rs10421768 is likely to be associated with HAMP promoter functions and that the A>G substitution may predispose to increased iron levels in beta-thalassemia patients. Also, this hypothesis was confirmed byZarghamian et al. [20] Which showed that the GG genotype was associated with increased levels of iron in the heart of patients with beta-thalassemia who did not respond to iron chelation therapy. The hypothesis that when the A>G nucleotide is substituted, there is a significant decrease in HAMP gene transcription due to the impaired attachment of transcription factors from the E-Box in the promoter area, is also confirmed. However, different results were obtained by Parajes, who performed an analysis of the effect of genetic variants on HAMP expression in vitro, and judged that the A>G variant only led to a slight decrease in its expression and found no significant relationship between the presence of homozygous GG and iron concentration serum transferrin levels and saturation in a Galician population [18].

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